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Yeast derivatives (extracts and autolysates) in winemaking: Release of volatile compounds and effects on wine aroma volatility

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Abstract

A qualitative study of volatile compounds in three commercial yeast extracts and autolysates was performed by solid-phase microextraction-gas chromatography with mass spectrometric and olfactometric detection; their addition to white wines and their effect on wine aroma composition were investigated by analytical, olfactometric and sensory evaluations. More than 160 volatile compounds were detected in the headspace of the commercial powders (some not previously reported in literature), and their olfactory characters were described. Yeast derivatives strongly modified wine aroma composition, either affecting the volatility of wine aroma compounds or by releasing exogenous volatiles. Dosage appeared to be fundamental: low amounts increased the volatility of some esters, giving more flowery and fruity notes to the wine; higher amounts increased fatty acid content in the wine headspace, producing yeasty, herbaceous and cheese-like smells. Sensory tests demonstrated that yeast derivatives would not be suitable for wines with a typical varietal aroma.

Keywords: Yeast extracts; Yeast autolysates; Wine; Volatile compounds; Solid-phase microextraction; Olfactometry; GC-MS

1. Introduction

Yeast macromolecules play a fundamental role in the colloidal equilibrium of wines. Mannoproteins, released during alcoholic fermentation and yeast autolysis, have been particularly studied in the recent years for their ability to improve tartaric stability (Lubbers, 1993; Lubbers, Leger, Charpentier, & Feuillat, 1993) and reduce the occurrence of protein hazes (Ledoux, Dulau, & Dubourdieu, 1992; Waters, Wallace, Tate, & Williams, 1993). Moreover, different authors have reported the role of polysaccharides and yeast macromolecules in stabilizing red wine color and phenolic compounds (Feuillat, Escot, Charpentier, & Dulau, 2001; Fuster & Escot, 2002; Saucier, Glories, & Roux, 2000; Saucier, Roux, & Glories, 1996).

For these reasons, one of the main goals of enological research in recent years was to develop commercial formulates of mannoproteins as stabilizing agents and technological adjuvants in winemaking (Feuillat, Charpentier, & Nguyen Van Long, 1998; Moine Ledoux, Perrin, Paladin, & Dubourdieu, 1997).

In the European Union, the main problem related to the use of mannoproteins as enological adjuvants is a legislative one: mannoproteins were approved by the Organisation Internationale de la Vigne et du Vin in 2001 (Resolution ŒNO 4/2001), but, under EU law, their use is currently permitted only for experimental trials (EU Regulation 1622/2000, art. 41).

This limitation forced enologists to look for alternatives, such as yeast derivatives, extracts and autolysates. These products are obtained from yeasts by autolytic, plasmolytic or hydrolytic processes, and then

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concentrated or dried to obtain the commercial formulates (Münch, Hofmann, & Schieberle, 1997; Nagodawithana, 1992). They recently appeared in the enological trade under different names (e.g., yeast walls, aging adjuvants), but their effects on wine composition are still not well understood.

The use of yeast derivatives in enology derived from food industry, where these products are widely used as flavoring agents (Nagodawithana, 1992), to simulate meat-like, broth-like or cheese-like flavors, and to aromatize snacks, soups and cheese products (Münch et al., 1997).

Up to now, only a few studies were performed on the volatile fraction of yeast extracts and autolysates (Ames & Elmore, 1992; Ames & McLeod, 1985; Münch et al., 1997; Münch & Schieberle, 1998; Werkhoff et al., 1991), and there are no scientific papers reporting their use in winemaking, or investigating the release of volatile compounds in wine.

However, the use of these products is well developed in enological practice, and their effects on wine aroma are not related just to their direct flavoring action. In fact, yeast derivatives, particularly autolysates, are not completely soluble, and the presence of particulates can be observed when they are added to wines; this insoluble fraction is composed of yeast cell wall residues that remain in the growth medium after the lysis treatment, and their ability to bind aroma compounds is well reported in literature (Lubbers, Charpentier, Feuillat, & Voilley, 1994a). Moreover, yeast macromolecules and colloids, released in wine during autolysis, can also determine different sensory effects, interacting with aroma compounds and modulating their volatility and perception (Lubbers, Voilley, Feuillat, & Charpentier, 1994b); yeast derivatives are used as a source of mannoproteins in winemaking, and could affect the aroma intensity of treated wines.

On the basis of these considerations, the aim of this study was to evaluate how industrial yeast derivatives can affect wine aroma perception. Increasing amounts of three different commercial yeast extracts and autolysates purchased in the trade were added to a white wine to simulate their enological use.

A qualitative screening of volatile compounds in the headspace of the commercial powders was performed by solid-phase microextraction-gas chromatography, with mass spectrometric (SPME-GC-MS) and olfactometric detection (SPME-GC-O). The effects of their addition on the volatility of wine aroma compounds, and the release of volatile compounds from the powders into the wine were investigated coupling SPME with GC-MS, GC-O and GC-flame ionization detection (FID).

Finally, the impact of the treatment on global aroma perception was evaluated for different white wines by sensory analyses.

2. Materials and methods

2.1. Chemicals

The following compounds were purchased from Sigma–Aldrich (St. Louis, MO, USA): acetaldehyde, 3-methylbutanal, 2,3-butanedione, 2,3-pentanedione, hexanal, 1-butanol, limonene, 1-pentanol, 3-hydroxy-2-butanone, 1-hexanol, ethyl octanoate, acetic acid, 2-furaldehyde, 2-ethyl-1-hexanol, 2-acetylfuran, benzaldehyde, propanoic acid, 1-octanol, 2-methylpropanoic acid, 5-methyl-2-furaldehyde, γ-butyrolactone, butanoic acid, ethyl decanoate, 1-nonanol, ethyl benzoate, 3-methylbutanoic acid, hexanoic acid, 2-methoxyphenol, 1-dodecanol, benzyl alcohol, 2-phenylethanol, benzothiazole, heptanoic acid, 2-methylphenol, octanoic acid, 4-methylphenol, (*R*)-dihydro-3-hydroxy-4,4-dimethyl-2(3*H*)-furanone, ethyl palmitate, decanoic acid, benzoic acid, dodecanoic acid.

2.2. Wine and yeast derivative samples preparation

Three commercially available yeast derivatives were used for the experimental trials; their characteristics are reported in Table 1: yeast extract (product E) and

Table 1
Characteristics and identification codes of yeast derivative samples

Sample	Identification code	Solubility[A,a]	Soluble proteins[A,b] (mg g^{-1} DM[f])	Soluble colloids[A,c] (mg g^{-1} DM)	Total nitrogen[d] (mg g^{-1} DM)	Total lipids[e] (mg g^{-1} DM)
Extract	E	+++	78 ± 3	462 ± 40	695 ± 21	85 ± 20
Autolysate 1	A	++	33 ± 2	282 ± 15	577 ± 2	121 ± 20
Autolysate 2	S	+	67 ± 2	386 ± 35	615 ± 6	150 ± 20

Numerical values are means and standard deviations of three repetitions.
[A] In hydroalcoholic solution (ethanol 10% v/v; pH 3.2).
[a] Evaluated on a visual base: +++ totally soluble; ++ low amount of particulate matter; + high amount of particulate matter.
[b] Lowry method, as reported by Regenstein and Regenstein (1984).
[c] Determined by ethanol precipitation (Usseglio-Tomasset & Castino, 1975).
[d] Kjeldahl method.
[e] Extracted by chloroform/methanol 2:1, v/v (D'Agostino, 1990).
[f] DM: dry matter.

autolysate 1 (product A) were purchased from Bio Springer (Maisons Alfort, France); autolysate 2 (product S), was from Pascal Biotech (Paris, France). All three samples were prepared from *Saccharomyces cerevisiae*. As regards products A and E, yeasts were grown on beet molasses containing media; autolysis was performed by enzymatic treatment and the autolysates dried to obtain the commercial powders (information supplied by the manufacturer). No information was given by the producer for product S.

To analyze the headspace of the commercial powders, 5 g of each product were closed in a 100 ml glass vial and immediately analyzed by SPME-GC-MS and SPME-GC-O.

For wine sample preparation, increasing amounts (200, 500, and 1000 mg l^{-1}) of yeast derivatives were added to a Chardonnay wine (2002, DOC Grave del Friuli, Italy): powders were weighed into 100 ml glass bottles and the wine was then drawn from a 5 l bulk, pumping it by a nitrogen flow to avoid oxidation phenomena. Bottles were closed with a crown cap closure, and stored for two weeks at 15 °C without stirring. All treatments were replicated three times, and a control wine without product addition was considered as reference sample.

Bottle contents were then homogenized by manual stirring. The presence of particulate matter was observed at the bottom of all the samples, and this was eliminated by overnight sedimentation after the stirring. For chromatographic analyses, 50 ml glass vials were filled with 25 ml of each sample. A nitrogen flow was again used to minimize oxidation phenomena and environmental pollution (Tat, Comuzzo, Stolfo, & Battistutta, 2005): nitrogen was blown inside the vial, before filling up, and a laminar flow was kept at its neck during filling in. Vials were then fitted with a silicone septum and stored at 15 °C until analysis.

Sensory tests were performed on three white wines: Pinot gris, Traminer (both from the DOC Grave del Friuli Region, 2002, Italy) and Sauvignon (2002, DOC Colli Orientali del Friuli, Italy); the first one was chosen for its neutral sensory character (as a non-aromatic white wine), the others for their typical varietal aroma.

Wines were drawn directly from the storage tanks after the fining treatments and just before bottling, filling 1 l glass bottles; autolysate 2 (product S in Table 1) was added (150, 300, 450 and 600 mg l^{-1}), and the bottles were closed with a crown cap closure. A reference sample without product addition was prepared as control.

Bottles were stored at 15 °C for two weeks and then homogenized (manual stirring); particulate matter was separated by sedimentation as reported above. Wines were racked (by nitrogen flow) in 0.75 l crown-capped bottles, and stored at 15 °C until tasting.

2.3. SPME sampling conditions

A 2 cm 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane fiber (Supelco, Bellefonte, PA, USA) was used for headspace analyses of both wines and yeast derivatives. As regards wines, SPME was run at 12 and 37 °C, for 15 min: these temperatures were chosen to simulate either the serving temperature of white wines (12 °C), or mouth temperature during tasting (37 °C). For yeast derivatives, SPME was performed only at 37 °C for the same time.

In both cases, a suitable conditioning system was used: the vials were dipped in a glass interspaced beaker filled with distilled water and connected with a thermostatic water bath (Model BT10D, Gibertini, Milan, Italy); the water flowed in the hollow space from the thermostatic bath, heating the water inside the beaker and providing the vial with thermostatation. For wine analyses, the beaker was put on the plate of a magnetic stirrer and provided with a magnetic stirring bar moving synchronically with another one placed into the vial; the first bar supplied thermostatation water with movement, the second provided the sample with agitation. No stirring bar was put in the vials of yeast derivative samples.

Vials were kept in the water bath for 15 min before SPME to reach thermal equilibration (Tat et al., 2005). SPME was immediately followed by GC injection.

2.4. GC-FID and GC-MS analysis

GC-FID analyses were performed using a Carlo Erba (Milan, Italy) HRGC 8560 Mega Series 2 gas chromatograph equipped with a flame ionization detector (FID) set at 240 °C. GC-MS analyses were carried out on a Varian (Palo Alto, CA, USA) 3400 gas chromatograph coupled to a Varian Saturn ITDMS ion trap mass spectrometer.

Both GC systems were provided with a split–splitless injection port, set at 260 °C. The carrier gas was helium, at a linear flow rate of 23 cm s^{-1}. Compounds were separated on an Econo-Cap Ec-Wax capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness), purchased from Alltech (State College, PA, USA). The column temperature was programmed as follows: 40 °C for 5 min, then at 4 °C min^{-1}, up to 240 °C, with a final holding time of 15 min. Injection was performed in splitless mode (70 s of splitless time); the fiber remained in the injector for the whole period of the splitless time.

For MS system, the temperatures of the manifold and transfer line were 170 and 250 °C, respectively; electron impact mass spectra were recorded at 70 eV ionization voltage, and the ionization current was 10 μA.

The identification of compounds was carried out by comparison of their mass spectra and retention times with those of standard compounds, or by comparison

of mass spectrum, with those reported in the mass spectrum library Wiley 5; moreover Kováts' retention indexes were calculated from the retention times of *n*-alkanes, and order of elution was compared with those available in literature (Ames & McLeod, 1985; Baek & Cadwallader, 1999; Jennings & Shibamoto, 1980; Lopez, Ferreira, Hernandez, & Cacho, 1999; Münch et al., 1997; http://www.nysaes.cornell.edu/flavornet/chem.html.).

2.5. GC-O analysis

The headspace of both wines and yeast derivatives was analyzed by GC-O using a Carlo Erba (Milan, Italy) HRGC 8560 Mega Series 2 gas chromatograph equipped with a FID system, and with an olfactometric detector, both connected with a flow splitter to the column end.

The column and chromatographic conditions were the same as those reported for GC-FID analysis. An additional make-up flow of nitrogen (21 ml min^{-1}) was supplied to improve the performances of the sniffing system, and an air flow (100 ml min^{-1}), humidified by bubbling in a distilled water reserve, was blown at the exit of the sniffing line, to cool and humidify the column flow.

2.6. Sensory tests

Two sensory tests were performed. The first was a Preference Test: a 10 member panel (6 enologists and 4 consumers) was called to test the wines and express an order of preference on the basis of a hedonic judgment.

The same panel took part in an Attribute Difference Test, to evaluate the impact of the dosage on wine sensory profile by the quantification (on a 0–10 scale) of different attributes connected with yeast derivatives technology: aroma intensity (to evaluate the effects of the treatment on the olfactory characters of the wines), aroma persistency (to evaluate the effects on retro-nasal perception), smoothness sensation (a factor connected with the release of macromolecules and colloids), and yeast-like aroma (to evaluate a direct impact of the products addition on wine aroma).

For both tests, wines were given in randomized order, and for the Attribute Difference Test, a sample was replicated for Pinot gris (600 mg l^{-1} of autolysate addition), to evaluate the repeatability of panelists.

2.7. Statistical analysis

A one way Analysis of Variance (ANOVA) was carried out on the absolute areas, detected by SPME-GC-FID analysis of the headspace of wine samples; means and standard deviations were calculated, and significant differences were evaluated by the Tukey Honest Significant Difference (HSD) Test. Variances were homogeneous according to Levene and Brown–Forsyte Tests; results were considered significant at $P < 0.05$.

The same approach (one way ANOVA) was used to investigate the effect of the dosage, as regards the Attribute Difference Test, working on the numerical values collected for each attribute. The judgments of each panelist were considered as replicates for the same sample.

Preference Test data were elaborated by Friedman test, as reported by the Barillere and Benard (1986), to evaluate the minimum significant difference between the ranks; the higher the sum of the ranks, the lower was the preference expressed by the panel.

Finally, Correspondence Analysis was used to study the relationship between dosage, preference, and mean values of the judgments collected for each attribute in the Attribute Difference Test. In this evaluation, panel preference was expressed as an additional attribute (preference index), calculated on the basis of the sum of the ranks, as determined for each sample by the Preference Test data elaboration. The sum of the ranks was subtracted from a theoretical value of 50 (i.e., the maximum value a sample could reach, if all 10 judges put that sample in the last position as regards preference), and then referred to a 0–10 scale by a simple proportion; in this way it was possible to find an index that was directly related to the preference of the panel.

All the statistical evaluations were performed using the specific software Statistica for Windows (StatSoft, Tulsa, OK, USA), Version 6.0.

3. Results and discussion

3.1. Qualitative characterization of the headspace of commercial powders

The results of SPME-GC-MS and SPME-GC-O analyses of the commercial formulates are reported in Table 2; 164 volatile compounds were detected in the headspace of the analyzed products: most of them were previously reported as yeast derivative volatile components, and their olfactory characters were already described (Ames & Elmore, 1992; Ames & McLeod, 1985; Münch et al., 1997; Münch & Schieberle, 1998).

Nevertheless, there are some differences in Table 2 between the odor descriptions reported in the literature and those detected by olfactometric analysis. This was probably due to the strong complexity of the headspace of the analyzed products, which led to very rich chromatographic profiles; as a result, it was quite difficult to associate a single label to a single compound in the description of the perceived odors, except for the most representative volatiles (such as carboxylic acids); more often, the odor perception connected with a single peak,

Table 2
Volatile compounds (SPME-GC-MS analysis) in the headspace of commercial yeast derivative samples; odor detection of impact odorants by SPME-GC-O

	Compound*	*I* Ref.[a]	*I*	*IM***	Odor description (literature)[a]	Odor detection by SPME-GC-O analysis	*CP*[k]
1	Acetaldehyde (ethanal)	690[c]	n.d.[l]	MS,S	Pungent[g]		S
2	Butyraldehyde (butanal)	1027[g]	n.d.	MS	Pungent[g]	Stale, mould	E
3	2-Butanone[i]	908[b,c]	949	MS,RI	Toffee[b]	Yeast, broth	S E
4	2-Methylfuran[i]	866[b]	952	MS,RI	Meat extract[b]		S
5	2-Methylbutanal[i]	1001[g]	990	MS,RI	Cat's urine[b]		A S
6	3-Methylbutanal[i]	937[b] 930[d]	996	MS,RI,S	Solvent[b] Malt[d]		A E S
7	Ethanol	929[g]	n.d.	MS	Sweet[g]		A E S
8	2-Pentanone	983[g]	980	MS,RI	Ethereal[g]	Pungent	S
9	Valeraldehyde (pentanal)	1002[c]	968	MS,RI	Pungent[g]		A E S
10	2,3-Butanedione (diacetyl)[i]	963[b,e] 970[g]	970	MS,RI,S	Butter[b]		S
11	2-Methylpentanal		973	MS			A
12	Acetonitrile		985	MS			E S
13	Propionitrile		1025	MS			E
14	Toluene[i]	1042[g]	1029	MS,RI	Paint[g]	Fruity, raspberry	A E S
15	2,3-Pentanedione[i]	1044[b]	1060	MS,RI,S	Creamy[g], roasty[b]		E S
16	Dimethyl disulfide (methyl disulfide)[i]	1081[b,c]	1064	MS,RI	Meat extract[b]		E
17	2-Hexanone		1079	MS			S
18	Caproaldehyde (hexanal)	1084[c]	1080	MS,RI,S	Cut grass[g]		A E S
19	2-Methyl-2-butenal		1086	MS			S
20	1-Methoxy-2-propanol		1115	MS			A E
21	2-Methoxyfuran		1127	MS			E
22	A dimethylbenzene (*m*- or *p*-xylene)		1128	MS,RI			S
23	Ethylbenzene		1130	MS		Ethereal, solvent	A E S
24	1-Butanol[i,h]	1113[b,c]	1132	MS,RI,S	Medicine[g]		A E S
25	2-Methylthiophene[i]	1123[b]	1124	MS,RI	Sulfur[g]		A S
26	1-Penten-3-ol	1157[g] 1130[c]	1157	MS,RI	Butter[g]		S
27	A terpene (β-pinene or sabinene)[i]		1159	MS,RI			A
28	2-Heptanone[i,h]	1172[b]	1167	MS,RI	Soap[g]		A E S
29	1,2-Dimethylbenzene (*o*-xylene)	1191[c]	1168	MS,RI	Geranium[g]		S
30	Pyridine	1180[c]	1180	MS,RI			S
31	Heptanal	1186[c]	1182	MS,RI	Fatty[g]	Yeast, cheese	A E S
32	Limonene[i,h]	1206[b]	1186	MS,RI,S	Lemon[g]		A E S
33	Pyrazine	1194[c]	1206	MS,RI			E S
34	2-Methylpyridine		1211	MS			S
35	2-Ethoxyethanol		1213	MS			A E
36	1-Ethyl-4-methylbenzene		1214	MS			A E S
37	1-Propen-2-ol (isopropenyl) acetate		1217	MS			E
38	2-Pentylfuran[i]	1229[b,c]	1228	MS,RI	Urine, rubbery[b]		A E S
39	2-Methylthiazole	1256[c]	1230	MS,RI			S
40	Thiazole[i]	1246[b,c]	1238	MS,RI			E S
41	1-Pentanol (amyl alcohol)[i,h]	1213[b]	1240	MS,RI,S	Balsamic[g]		A E S
42	1,2,4-Trimethylbenzene (pseudocumene)		1246	MS			A
43	2-Methylpyrazine[i]	1251[b,c]	1260	MS,RI	Popcorn[g]	Pepper, sweet	A E S
44	1-Methyl-4-isopropylbenzene (*p*-cymene)	1272[c]	1266	MS,RI	Solvent[g]		A E
45	1,2,3-Trimethylbenzene		1273	MS			A E
46	2-Ethylpyridine		1277	MS			S
47	3-Hydroxy-2-butanone		1277	MS,S		Stale, mould	E S
48	2-Octanone	1285[g]	1278	MS,RI	Soap[g]		E S
49	Octanal	1278[c] 1296[f]	1290	MS,RI	Unpleasant[f]		A E S

(*continued on next page*)

Table 2 (*continued*)

	Compound*	*I* Ref.[a]	*I*	*IM***	Odor description (literature)[a]	Odor detection by SPME-GC-O analysis	*CP*[k]
50	3-Methylpyridine		1294	MS			S
51	2,5-Dimethylpyrazine[i]	1306[b,c]	1320	MS,RI	Peanut butter, solvent[g]	Herbaceous, pungent, cabbage, potato	E S
52	2-Ethyl-1,4-dimethylbenzene		1325	MS			A
53	2,6-Dimethylpyrazine[i]	1325[b,c]	1325	MS,RI	Peanut butter, solvent[g]		S
54	2-Ethylpyrazine[i]	1387[b,c]	1330	MS,RI			S
55	6-Methyl-5-hepten-2-one	1336[g]	1334	MS,RI	Pungent[g]		A E S
56	2,3-Dimethylpyrazine	1330[c]	1348	MS,RI			S
57	4-Ethyl-1,2-dimethylbenzene		1352	MS			A E S
58	2-Isopropylpyrazine		1354	MS		Herbaceous, pungent, cabbage, potato	S
59	1-Hexanol	1316[c] 1359[f]	1357	MS,RI,S	Herbaceous, resinous[g]		A E S
60	4-Hydroxy-4-methyl-2-pentanone		1359	MS			A E S
61	2-Ethyl-6-methylpyrazine[i]	1425[b]	1384	MS,RI	Rancid, solvent[b]	Unpleasant, pungent	S
62	2-Ethyl-5-methylpyrazine[i]	1425[b]	1388	MS,RI	Rancid, solvent[b]		S
63	Nonanal	1382[c]	1391	MS,RI	Soap[g]	Unpleasant, pungent	A E
64	2-Butoxyethanol		1407	MS		Unpleasant, pungent	A E S
65	2-Ethyl-3-methylpyrazine[i]	1381[b]	1407	MS,RI			S
66	2,3,5-Trimethylpyrazine[i]	1387[b]	1408	MS,RI			S
67	2-Methyl-5-isopropylpyrazine		1411	MS			S
68	A tetramethylbenzene		n.d.	MS		Potato	E S
69	3-Methyl-3-cyclohexen-1-one		1417	MS			A S
70	1,3-Bis(1,1′-dimethylethyl)benzene		1431	MS			A E
71	Ethyl caprylate (octanoate)[i,h]	1435[e]	1437	MS,RI,S	Floral, fruity[g]		A E
72	Acetic acid[j,h]	1451[e] 1436[d]	1446	MS,RI,S	Sour, pungent[d,g]	Sour, vinegar	A E S
73	3-Ethyl-2,5-dimethylpyrazine[i]		1464	MS,RI	Potato[g] smoky, burnt[b]	Herbaceous, pungent	S
74	A diethylpyrazine[i]	1455[b]	1468	MS,RI			S
75	2-Furaldehyde (furfural)[i,h]	1449[b,c]	1470	MS,RI,S	Oily, rancid[b]	Cheese, pungent	A E S
76	2,3,5,6-Tetramethylpyrazine[i]	1458[b]	1472	MS,RI	Unpleasant[b]	Yeast, cooked vegetables, mould	S
77	2-Methyl-3-propylpyrazine	1462[c]	1474	MS,RI			S
78	1-(2-Methoxy-1-methoxyethoxy)-2-propanol		1494	MS			A E S
79	3,5-Diethyl-2-methylpyrazine		1498	MS			S
80	2-Ethyl-1-hexanol[h]	1380[g]	1500	MS,S	Rose[g]		A E S
81	Decanal	1485[c]	1504	MS,RI	Soap, resinous[g]		A
82	2-Acetylfuran[i]	1491[b,c]	1507	MS,RI,S	Floral, honey[b]		S
83	Camphor	1491[g] 1518[c]	1508	MS,RI	Camphor[g]		E
84	2,3-Diethyl-5-methylpyrazine[j]	1481[d]	1511	MS,RI	Roasted potato[d]		S
85	Formic acid[i]		1525	MS,RI			E
86	Benzaldehyde[i,h]	1502[b,c]	1528	MS,RI,S	Almond[b]		A E S
87	Propanoic acid[j]	1528[d]	1540	MS,RI,S	Cheese[d]	Cheese	A E S
88	1-Octanol[h]	1519[c]	1568	MS,RI,S	Solvent[g]		A E S
89	2-Methylpropanoic acid[i,j,h]	1548[d]	1574	MS,RI,S	Cheese[d]	Pungent	A E S
90	5-Methyl-2-furaldehyde[i]	1563[b,c]	1578	MS,RI,S	Fatty, roasty[b]		A
91	2,2-Dimethylpropanoic acid		1594	MS		Herbaceous	A E S
92	3,5,5-Trimethyl-2-cyclohexen-1-one (isophorone)[i]		1602	MS,RI			E S
93	Dihydro-5-methyl-2(3*H*)-furanone (γ-valerolactone)	1617[c]	1609	MS,RI			A E S
94	Terpinen-4-ol (4-carvomentenol)[i]	1628[b]	1632	MS,RI	Stale, mould[g]		S
95	Dihydro-2(3*H*)-furanone (γ - butyrolactone)[h]	1632[c]	1634	MS,RI,S	Caramel[g]		A E S
96	Butanoic acid[i,j,h]	1612[d]	1638	MS,RI,S	Cheese[d]	Cheese	A E S
97	Ethyl caprate (decanoate)[h]	1634[f]	1659	MS,RI,S	Grape[g] soap[f]	Yeast, broth	S
98	2-acetylthiazole[j]	1639[c] 1620[d]	1662	MS, RI	Roasty[d,g]		S
99	1-Phenylethanone (acetophenone)	1627[c]	1663	MS,RI			E S
100	3-(3-Methylbutyl)-2,5-dimethylpyrazine		1668	MS			S
101	1-Nonanol	1624[c]	1673	MS,RI,S			A
102	2-Furanmethanol (furfuryl alcohol)[i]	1673		MS,RI	Burnt[g]		E S

Table 2 (*continued*)

	Compound*	*I* Ref.[a]	*I*	*IM***	Odor description (literature)[a]	Odor detection by SPME-GC-O analysis	*CP*[k]
103	Ethyl benzoate	1647[c]	1675	MS,RI,S	Fruity[g]		A
104	isovaleric acid (3-methylbutanoic)[j,h]	1651[d] 1672[e]	1677	MS,RI,S	Cheese[f] sweet[d]	Cheese	A E S
105	2-Methyl-2-pentenal		1703	MS			A
106	4-Hydroxy-2-methylenbutanoic acid		1704	MS		Cheese	A S
107	2-Acetylthiophene[i]	1760[b]	1709	MS,RI			A E S
108	A thiophene (acetyl or methyl)		1712	MST			S
109	2-Pentanoylfuran	1747[c]	1726	MS,RI			S
110	Valeric acid (pentanoic)[i]	1723[d]	1750	MS,RI	Sweet[d,g]	Pungent, herbaceous	A E S
111	2-Methylpentanoic acid		1773	MS			A
112	A (1,1′-dimethylethyl)thiophene		1775	MST			E
113	Acetamide		1780	MS			A E S
114	1-Decanol[h]	1723[c]	1783	MS,RI	Fatty[g]		A E S
115	A sesquiterpene		1785	MST			E
116	2-Butenoic acid		1808	MS		Pungent, cheese	A E
117	Formamide		1812	MS			E S
118	2-Methylpropionamide (isobutyramide)		1824	MS		Pepper	E S
119	Propionamide		1827	MS			A E S
120	2-Methylbenzenamine (*o*-toluidine)		1830	MS			A
121	Caproic acid (hexanoic)[i,h]	1852[e,f]	1878	MS,RI,S	Cheese[f]	Cabbage	A E S
122	(*Z*)-6,10-dimethyl-5,9-undecadien-2-one (geranylacetone)	1796[g]	1888	MS	Magnolia[g]		E S
123	2-Methoxyphenol (guaiacol)[i j]	1840[b,d]	1891	MS,RI,S	Burnt[d,g]		A E S
124	1-Dodecanol	1925[c]	1895	MS,RI,S	Waxy[g]		A E
125	Benzyl alcohol[i,h]	1822[b]	1900	MS,RI,S	Sweet[g]		A E S
126	Butanamide		1919	MS			E
127	Diacetamide		1926	MS			E S
128	2-Phenylethanol[i,j,h]	1859[b] 1898[d]	1934	MS,RI,S	Floral[d]		A E S
129	3-Methylbutanamide		1935	MS			E S
130	2,6-Bis(1,1′-dimethylethyl)-4-methylphenol (BHT)		1936	MS			E S
131	1,4-Butandiol	1861[c]	1954	MS			A E S
132	Benzothiazole[h]	1588[g]	1972	MS,S	Rubbery[g]		E S
133	Heptanoic acid[h]		1974	MS,S			A E S
134	2-Methoxy-4-methylphenol (4-methylguaiacol, creosol)		1977	MS			S
135	2-Acetylpyrrole[i]	1935[b]	2000	MS,RI		Pungent, sour	A E S
136	2-Methylphenol (*o*-cresol)	2017[g]	2037	MS,RI,S	Phenolic[g]		A E S
137	5,6-Dihydro-4-methyl-2*H*-pyran-2-one		2042	MS			E S
138	2-Pyrrolidinone		2045	MS			A E S
139	(*R*)-dihydro-3-hydroxy-4,4-dimethyl-2(3*H*)-furanone (pantolactone)		2050	MS,S		Yeast	A E S
140	1-Phenoxypropan-2-ol		2080	MS			A E
141	Caprylic acid (octanoic)[i,h]	2060[f]	2092	MS,RI,S	Rancid[f]	Stale, mould	A E S
142	4-Methylphenol (*p*-cresol)	2067[g]	2123	MS,S	Medicine[g]		S
143	A phenol		n.d.	MST		Stale, mould	E S
144	A thiophene		n.d.	MST			E S
145	2-Phenoxyethanol		2182	MS			E
146	2,6-Diisopropylnaphthalene (or a derivative)		2182	MS			S
147	A dibutylnaphthalene		2212	MS			E
148	Nonanoic acid[h]	2110[g]	2216	MS	Cheese[g]		A E S
149	3-Ethylphenol	2150[c]	2221	MS			S
150	2-Methyl-5-isopropylphenol (carvacrol)	2159[c]	2261	MS			E S

(*continued on next page*)

Table 2 (*continued*)

	Compound*	*I* Ref.[a]	*I*	*IM***	Odor description (literature)[a]	Odor detection by SPME-GC-O analysis	*CP*[k]
151	Methyl palmitate (hexadecanoate)	2204[c]	2293	MS			A E
152	2-Benzothiazolecarboxaldehyde		2297	MS			S
153	Ethyl palmitate (hexadecanoate)[h]	2251[g]	2300	MS,S	Waxy[g]		A
154	2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one		2305	MS			A E
155	Capric acid (decanoic)[i,h]	2229[f]	2309	MS,RI,S	Unpleasant[f]		A E S
156	4-Methyl-5-tiazolethanol	2216[c]	2342	MS			E S
157	2,4-Bis(1,1′-dimethylethyl)phenol		2353	MS			A E
158	Benzoic acid[h]		2487	MS,S			A E S
159	2,5-Pyrrolidinedione (succinimide)		2525	MS			A E S
160	Benzophenone	2410[c]	2533	MS			A E S
161	Lauric acid (dodecanoic)[i]	2504[g]	2538	MS,RI,S			A E S
162	A phthalate (dibutyl phthalate)[i]		2598	MS,RI			A E S
163	Myristic acid (tetradecanoic)[h]		n.d.	MS			A
164	Palmitic acid (hexadecanoic)[h]		n.d.	MS			A E S

I: Kováts' retention index. [a]Reported by different authors: [b]Ames and McLeod (1985) and [c]Jennings and Shibamoto (1980) on a Carbowax 20 M column; [d]Münch et al. (1997) on a FFAP column; [e]Baek and Cadwallader (1999) on a DBWax column;[f] Lopez et al. (1999) on a Carbowax 20 M column; [g]reported in http://www.nysaes.cornell.edu/flavornet/chem.html. *Compound: [h]also detected in the headspace of the Chardonnay wine utilized in the experimental trials; [i]previously reported as yeast derivatives volatile components by Ames and McLeod (1985) and [j]by Münch et al. (1997). **IM: Identification method:[S] comparison of mass spectra and retention time with those of standard compounds; [RI]comparison of order of elution according to different authors (Ames & McLeod, 1985; Baek & Cadwallader, 1999; Jennings & Shibamoto, 1980; Lopez et al., 1999; Münch et al., 1997; http://www.nysaes.cornell.edu/ flavornet/chem.html.); [MS]comparison of mass spectra with those reported in Wiley 5 mass spectrum library; [MST]tentative identification by mass spectrum. [k]CP: Commercial product in which volatile compounds were detected (see Table 1); [l]n.d.: not determined.

or with a given chromatographic region, was rapidly changed by the elution of the subsequent compounds.

Despite this superimposition of odorous sensations, some analogies can be found in Table 2 between the odors perceived in a given chromatographic zone (by SPME-GC-O analysis), and the odors reported in the literature for compounds that are eluted in that zone. For example, the descriptions previously reported for 2-pentanone and valeraldehyde, ''ethereal'' and ''pungent'', respectively (http://www.nysaes.cornell.edu/flavornet/chem.html.), are connected with the ''pungent'' sensation perceived by olfactometric detection; the labels ''soap'' and ''unpleasant'', that are given for 2-octanone (http://www.nysaes.cornell.edu/flavornet/chem.html.) and octanal (Lopez et al., 1999), could be described with the perceived unpleasant sensations; finally, 2-ethyl-6-methylpyrazine and 2-ethyl-5-methylpyrazine, connected with ''nutty'', ''rancid'' and ''solvent-like'' sensations (Ames & McLeod, 1985), were detected as ''cheese'' and ''unpleasant'' odors, and 2-methylphenol, previously connected to ''phenolic'' characters (http://www.nysaes.cornell.edu/flavornet/chem.html.), was described as a ''pungent'' smell.

The main observation about these olfactory characters, is that they are not typical odors for white wines, and they have certainly not a good relation with the freshness of wine aroma; more probably they could be connected to some characters of aged wines, particularly as concerns the ''yeast-like'' odors.

As regards the origin of these volatile compounds, a comprehensive study (Ames & McLeod, 1985) reported different hypotheses on their mechanisms of formation: some are biosynthesized by the yeasts, others originate from the thermal and oxidative degradation of lipids, but the main impact on the genesis of odorous compounds in yeast derivatives manufacture derives from the action of heat on sugars, amino acids, and thiamin (Ames & Elmore, 1992). These precursors are thermally degraded or interact (under the influence of heat) during the final drying process to obtain the powdered formulates. Working on both commercial and self-prepared yeast extracts, Münch and co-workers demonstrated a good correlation between the amounts of certain precursor amino acids, and the odor activities of some key odorants generated by thermal treatment (Münch et al., 1997; Münch & Schieberle, 1998).

So, temperature has a fundamental role in the development of yeast derivatives flavoring characters. Different papers (Davidek, Hajslova, Kubelka, & Velisek, 1979; Hajslova, Velisek, Davidek, & Kubelka, 1980) reported that heating (100 °C for 10 min) could determine a significant change in the overall odor of a yeast extract. In particular, the Maillard reaction is one of the main factors involved in the formation of yeast aroma; as regards the compounds reported in Table 2, 2-furaldehyde, pyrroles, pyrazines, pyridines, thiazoles and furans, detected in all the analyzed formulates, are well

known in literature as Maillard volatile products (Nagodawithana, 1992).

Strecker degradation of amino acids is another fundamental factor connected with the formation of strongly odorous compounds, such as some aldehydes: 2-methylbutanal (originated from isoleucine) and 3-methylbutanal (from leucine) were detected in all the commercial powders, and previously reported in different papers (Ames & McLeod, 1985; Münch et al., 1997); some carboxylic acids, such as 2-methylbutanoic, and 2-methylpropanoic, originate from the oxidation of Strecker aldehydes (Ames & McLeod, 1985).

A relatively low number of sulfur compounds (particularly thiophenes and thiazoles) are listed in Table 2, if compared with the results of other authors. Werkhoff et al. (1991) reported 115 sulfur compounds as yeast extract aroma components; some powerful key odorants identified in previous studies (Münch et al., 1997; Münch & Schieberle, 1998), such as 2-furanmethanethiol (roasty and coffee-like sensations), methional (cooked potato), 2-methyl-3-furanthiol (meat-like), or 3-mercapto-2-pentanone (sulfury), were not detected in the analyzed products.

Different volatiles reported in Table 2 appear to be connected with oxidative degradation of fats: alcohols (1-pentanol), carbonylic compounds (2-heptanone), 2-pentylfuran (Ames & McLeod, 1985; Vichi, Pizzale, Conte, Buxaderas, & López-Tamames, 2003), and some aldehydes (hexanal, octanal, nonanal), show a good correlation with the oxidation of lipidic matrixes (Grosch, 1987; Vichi et al., 2003); some carboxylic acids (C8-C16), γ- and δ-lactones are related with the thermal degradation of fats (Nawar, 1969).

The presence of volatile phenols (such as guaiacol) is usually connected with the addition of spices and vegetable extracts in the manufacture of yeast derivatives. They could derive from lignin and malt phenolic precursors (ferulic acid), and for this reason, could be also related to the use of brewers' yeasts as starting material (Ames & McLeod, 1985). Nevertheless, guaiacol was detected in all the analyzed yeast extracts and autolysates, i.e., in products obtained starting from *Saccharomyces cerevisiae*, appositely grown for this purpose.

Different compounds reported in Table 2 are well known as contaminants in foods and beverages. In particular benzene derivatives (toluene, and alkyl benzenes) are commonly detected in our laboratories in different foodstuffs: wine, olive oils, coffee, spices, honey and cheese; their classification as environmental pollutants is widely reported (Fabietti, Delise, & Piccioli Bocca, 2000; Page & Lacroix, 2000). Finally, the presence of BHT [2,6-bis(1,1′-dimetyletyl)-4-metylphenol] in autolysate 2 (product S), could be due to its use as an anti-oxidant, but also to its release from the plastic packaging (Tombesi & Freije, 2002).

Some compounds, detected in the commercial products, and previously reported in the literature, are commonly known as wine volatile components: these are terpenes (limonene), alcohols (1-butanol, 1-hexanol, 2-phenylethanol), carboxylic acids (hexanoic, octanoic), esters (ethyl octanoate) and carbonylic compounds (2-heptanone). Their amount in yeast derivatives volatile fraction seemed quite low, if compared with the chromatographic response (absolute area) that they gave in the analysis of wines; for this reason, a direct impact on wine sensory perception seems not very probable at the common dosages. To the contrary, carboxylic acids, such as acetic and butanoic, are some of the most representative constituents in the headspace of the analyzed powders (Ames & Elmore, 1992; see Fig. 1), so they could have a strong effect on wine aroma.

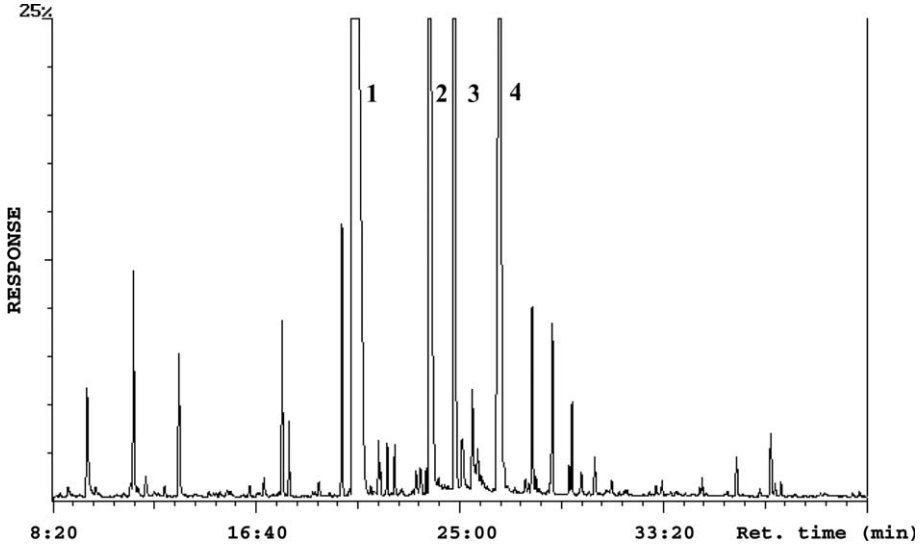

Fig. 1. Gas chromatogram of the headspace of a commercial yeast extract (product E) analyzed by SPME-GC-MS (recorded in full scan mode). (1) acetic acid; (2) propanoic acid; (3) 2-methylpropanoic acid; (4) butanoic acid.

Some volatile components, not previously reported in literature, were detected and tentatively identified in yeast derivative samples.

Amides were detected in all the tested powders. A possible explanation for their presence in yeast derivatives could be their strong relationship with the corresponding carboxylic acids (that were found in the commercial products): acetamide and acetic acid, butanamide and butanoic acid, 2-methylpropanamide and 2-methylpropanoic acid, 3-methylbutanamide and 3-methylbutanoic acid. Indeed, a possible method for the industrial synthesis of amides is the heating of carboxylic acids in the presence of ammonium salts (Streitwieser, Heathcock, & Kosower, 1992), so the genesis of these compounds in yeast derivatives manufacture could be related to the final drying process, if one considers the addition of ammonium salts as a nitrogen source for the yeasts. However, this hypothesis is not verified at this time and, in general, amides were not associated to odorous chromatographic regions.

On the contrary, strong potato-like and cabbage-like smells were perceived in three chromatographic zones, characterized by carbonylic compounds that were not previously detected in yeast extracts or autolysates: 6-methyl-5-hepten-2-one, 4-hydroxy-4-methyl-2-pentanone and 3-methyl-3-cyclohexen-1-one were tentatively identified in all the analyzed products.

The first one (6-methyl-5-hepten-2-one) is a breakdown product of carotenoids, as geranylacetone (Schreier, Drawert, & Junker, 1977) and isophorone (Piasenzotto, Gracco, & Conte, 2003), that were also detected in the commercial products.

Little evidence was found in literature as regards the mechanism of formation for 4-hydroxy-4-methyl-2-pentanone and 3-methyl-3-cyclohexen-1-one, and it is rather difficult to explain their presence in the analyzed products. They might derive from oxidative degradation of lipids (as other ketones), but 4-hydroxy-4-methyl-2-pentanone may originate from aldolic condensation mechanisms, starting from other carbonylic compounds (Streitwieser et al., 1992). Further study is required to clarify the formation of these odorants.

Finally, qualitative differences can be observed in the olfactometric profile of the three yeast derivatives and, as a consequence, in the global odor perception of the powders: the cheese-like and chips-like aroma of products A (autolysate 1) and E (yeast extract) could be mainly connected with the presence of carboxylic acids and aldehydes, while for product S (autolysate 2) the presence of pyrazines in the chromatogram could explain its strong broth-like and yeast-like smell. These differences in the overall aromatic pattern are fundamental, because a simple olfactory test would be a suitable tool to help the enologist in the choice of a good (more or less odorous) formulate.

3.2. Effect of the commercial products addition on the impact odorants of treated wines

As reported in the previous section, the aroma composition of the commercial formulates was particularly related to pungent, vegetal, yeast, and cheese-like odors; these sensory characters could also be marked in the olfactometric profile of the treated wines.

The impact odorants detected in the headspace of wine samples are reported in Table 3. This shows that the olfactometric profile of the Chardonnay (control wine) was strongly modified by the treatment and the odor perception of some volatile compounds was strongly affected by the dosage.

There are also analogies as regards the three yeast derivatives: the lowest additions (200 mg l^{-1}) generally determined the appearance of flowery and fruity notes that were not detected in the odorous profile of the control wine; these smells were connected with some volatile esters (ethyl octanoate, 2-phenylethyl acetate, isoamyl octanoate, ethyl myristate), alcohols and terpenes (linalool and 1-octanol, 1-decanol and β-citronellol); increasing the dosage, yeast-like, cheese-like and unpleasant notes appeared as new perceptions in the olfactogram; these odors seemed to be connected with some carboxylic acids, particularly butanoic, hexanoic, and decanoic acid.

As Table 3 shows, all volatile compounds that were detected as responsible for these unpleasant smells were also found (with similar odors) in the headspace of the commercial formulates (see also Table 2). This led us to think that they could be directly released from the powders into the wine.

This hypothesis was confirmed for some volatiles by SPME-GC-FID analysis. Fig. 2 reports the behavior of butanoic and decanoic acid contents (expressed in absolute area units) in the headspace of wine samples as a function of yeast extract and autolysate 1 addition: absolute areas were significantly increased ($P < 0.05$) by the addition of commercial formulates and this is evident for both compounds.

As regards decanoic acid, this behavior could easily be connected (for product A) to the herbaceous and cheese-like smells reported in Table 3 for the highest dosages, while for butanoic acid, it could be interesting to analyze some additional considerations.

Butanoic acid is one of the main components of yeast derivatives volatile fraction (see Fig. 1), and its unpleasant odor was detected by GC-O for the highest additions of all the commercial products (Table 3); nevertheless, ANOVA results marked significant differences only as regards yeast extract addition (Fig. 2), with the sensory perception connected to this carboxylic acid being particularly evident in the wines treated with this product.

Yeast extract was very soluble in hydroalcoholic solution, and this could determine an easier release of odorous compounds in the wines. Indeed, the wine samples

Table 3
Impact odorants detected in the headspace of Chardonnay wine samples (SPME-GC-O and SPME-GC-MS analyses), as a function of different amounts of yeast derivatives

R_t[a]	Chromatographic zones	Odor detection by SPME-GC-O analysis				CP[c]
	Compound	Yeast derivatives addition (mg l^{-1})				
		0[d]	200	500	1000	
6,07	Ethyl butanoate	Tropical fruits	Strawberry	Strawberry	Fruits	A E S
6,56	Ethyl 2-methylbutanoate	Tropical fruits		Fruits, sweet	Banana	A E S
7,06	Ethyl 3-methylbutanoate	Fruits	Fruits, sweet	Fruits, sweet		A E S
8,93	Isoamyl acetate	Banana	Fruits, sweet	Banana	Red fruits	A E S
9,36	1-Butanol[b]		Mould, pungent	Mould		A E
10,59	2-Heptanone[b]					
12,66	2- and 3-Methyl-1-butanol	Pungent	Pungent, cheese	Pungent, cheese	Pungent, cheese	A E S
13,26	Ethyl hexanoate (caproate)	Red fruits	Strawberry	Strawberry	Fruits	A E S
15,40	4-Methyl-1-pentanol		Mould (weak)		Ammonia, pungent	A S
16,50	3-Methyl-1-pentanol					
20,60	Ethyl octanoate (caprylate)[b]		Violet, flowers			A E S
21,53	2-Furaldehyde (furfural)[b]	Yeast, cabbage (weak)	Yeast (weak)	Cabbage	Cheese, pungent	A E S
23,90	2-Ethyl-1-hexanol[b]				Herbaceous	A
24,50	Linalool		Citrus fruits	Flowers	Solvent	A
24,70	1-Octanol[b]					
27,00	Butanoic acid[b]			Dung, unpleasant	Unpleasant	A E S
28,07	Isoamyl octanoate		Peach			S
28,42	3-Methylbutanoic acid[b]	Cheese (weak)	Cheese	Unpleasant	Animal, pungent	A E S
31,39	1-Decanol[b]		Fruits (weak)			E
31,70	β-Citronellol					
32,60	2-Phenylethyl acetate		Fruits (weak)	fruits		A E S
33,20	Hexanoic (caproic) acid[b]	Smoke		Mould	Herbaceous	A E S
34,79	2-Phenylethanol[b]	Strawberry, candy	Fruits			A E S
35,37	Benzothiazole	Herbaceous	Yeast, mould	Yeast, mould	Herbaceous, pungent	A E S
36,44	Heptanoic acid[b]					
39,00	Octanoic (caprylic) acid[b]		Burnt			E
39,16	Ethyl myristate (tetradecanoate)		Fruits	Fruits		A S
45,24	Decanoic (capric) acid[b]			Mould, cheese	Herbaceous, cheese	A E S

[a] R_t: retention time (min).
[b] Volatile compounds also detected in the headspace of the commercial powders.
[c] CP: Yeast derivatives (see Table 1).
[d] Control wine (no product addition).

treated with yeast extract (E) showed a statistically higher level of butanoic acid in their headspace, when compared with the same dosage of the less soluble autolysates (data not reported). The use of yeast autolysates with a low solubility could therefore be a useful tool to reduce the release of exogenous compounds from the powders into wine.

Contrarily to what was observed for carboxylic acids, the effect of dosage on the olfactometric perception of esters appeared to be more complex: for example, 2-phenylethyl acetate or isoamyl octanoate were not found in the headspace of yeast derivative powders and the appearance of their fruity odor in some of the treated wines is surely not explained by their direct release from the commercial formulates.

In a previous study (Comuzzo, 2003), we observed that the volatility of five typical wine aroma compounds was affected by the soluble colloidal fraction of a yeast autolysate in wine-like solution. Moreover, the effect of yeast macromolecules and colloids on the volatility of wine aroma compounds is well reported in literature (Dufour & Bayonove, 1999; Lubbers et al., 1994b; Voilley, Beghin, Charpentier, & Peyron, 1991; Voilley, Lamer, Dubois, & Feuillat, 1990). So, the behavior of the olfactometric perception of some esters reported in Table 3 could be connected to the ability of yeast derivatives to increase their volatility.

As regards product A, this hypothesis can be confirmed by the results of SPME-GC-FID analyses. Fig. 3 shows that the addition of 200 mg l^{-1} of the commercial autolysate determined a higher level of acetic esters in the headspace of Chardonnay wine samples. On the basis of ANOVA results, this behavior was particularly related to isoamyl acetate, hexyl acetate and (*Z*)-3-hexen-1-ol acetate, while ethyl acetate and 2-phenylethyl acetate seemed not to be statistically affected by the treatment. This last observation is rather strange, because in Table 3, 2-phenylethyl acetate was the only acetic ester affected by the treatment (as regards olfactometry).

These differences between FID and olfactometric detection could probably be related either to the concentration of these esters in the headspace of the analyzed

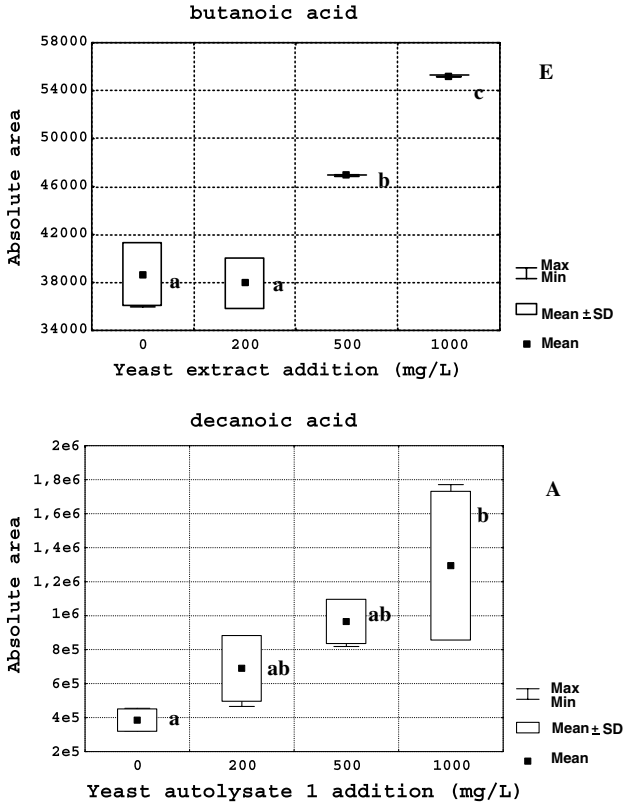

Fig. 2. Butanoic and decanoic acid content (absolute areas detected by SPME-GC-FID analysis) in the headspace of Chardonnay wine samples: effect of increasing additions of yeast extract (E) and autolysate 1 (A) respectively. SPME run at 37 °C. Different letters represent means which are significantly different at $P < 0.05$.

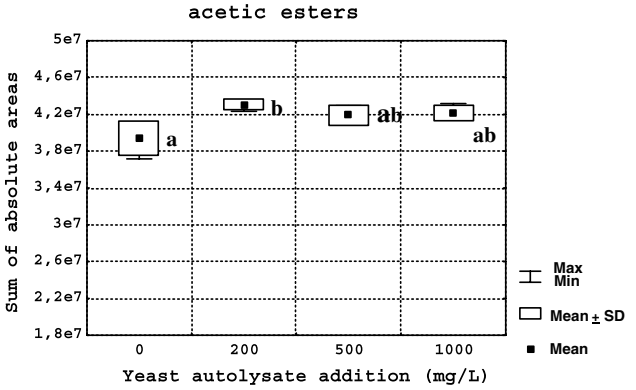

Fig. 3. Acetic esters[a] content (sum of absolute areas detected by SPME-GC-FID analysis) in the headspace of Chardonnay wine samples: effect of increasing additions of autolysate 1. SPME run at 12 °C. Different letters represent means which are significantly different at $P < 0.05$[a] Ethyl acetate; isoamyl acetate; hexyl acetate; (*Z*)-3-hexen-1-ol acetate; (*E*)-3-hexen-1-ol acetate; 2-phenylethyl acetate.

samples, or to their odor thresholds, particularly as regards difference thresholds. For this reason, a significant difference between two dosages, as detected by GC-FID analysis, would not be detectable by olfactometry, or vice-versa. For example, (*Z*)-3-hexen-1-ol acetate was not abundant in the Chardonnay (the absolute area values detected at 37 °C were the lowest if compared with those of the other acetic esters), and it was not detected in the olfactogram of the control wine; for this reason, its concentration in the wine seems to be lower than its odor threshold, and the significant differences marked by ANOVA for GC-FID analyses would not be detectable by GC-O.

On the basis of these observations, yeast derivatives and their effects on the volatile fraction of wines should be studied further, considering both the release of exogenous volatiles (and their quantitation in the commercial powders) and the effects of the treatment on wine aroma volatility, depending on aroma headspace concentration, odor thresholds and relative concentration.

As observed for esters, other compounds were affected by the lowest dosages of products A and E, resulting in some fruity notes that were not detectable in the control wine. This is particularly evident in Table 3 as regards the chromatographic regions of linalool and 1-octanol (product A), and 1-decanol and β-citronellol (product E). It seems logical that terpenes were mainly responsible for these odors, even if no significant differences were marked by SPME-GC-FID and ANOVA analyses as regards the behavior of these compounds in the headspace of the treated wines. It is also interesting to note that, in the same chromatographic zone, the citrus and flowery detection of linalool changed in a solvent perception, with the increase of the dosage of autolysate 1 (A); this odor is the same as that detected for 1-octanol in the commercial formulates (Table 2), confirming the possible release of this alcohol from the powders.

3.3. Sensory analyses results

A consequence of what was observed in the analytical evaluations is that the overall aroma perception of wines can be modified by the addition of yeast derivatives.

Thus, another question could be important to define the global effect of the treatment: are yeast derivatives suitable additives for any kind of wine? Indeed, the impact odorants reported in Table 2 are surely not desirable for wines characterized by typical varietal aroma.

This hypothesis was investigated by sensory evaluation of three different white wines treated with increasing amounts of product S; this product was chosen for its low solubility, to tentatively minimize the release of volatile compounds into the wines.

At a first analysis, no significant differences were found as a function of dosage, either by ANOVA, as regards the numerical values collected in the Attribute Difference Test, or by Friedman Test, as regards the Preference Test results.

Anyway, Correspondence Analysis highlighted some interesting observations. Fig. 4 reports the results for

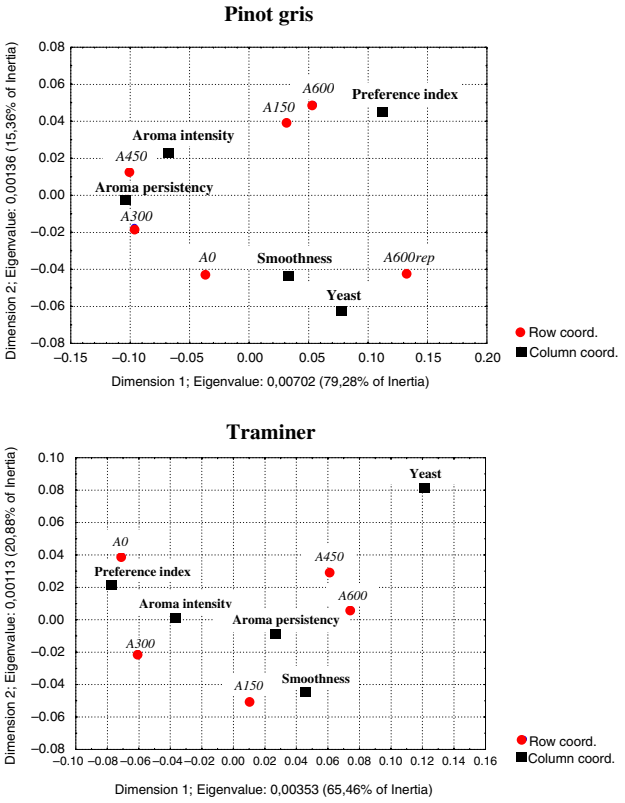

Fig. 4. Correspondence Analysis results: results of the sensory tests (Preference Test and Attribute Difference Test) performed for Pinot gris and Traminer wines after addition of increasing amounts of yeast autolysate 2: 150 mg l^{-1} (*A*150), 300 mg l^{-1} (*A*300), 450 mg l^{-1} (*A*450) and 600 mg l^{-1} (*A*600); *A*600rep: 600 mg l^{-1}, replicated sample; *A*0: control wine (no product addition).

Pinot gris and Traminer wines: if compared to those obtained by ANOVA and Friedman Test they are surely not exhaustive, but the observed trend suggests some useful practical considerations.

As regards Pinot gris, the preference of the panel was quite close to the samples treated with 150 and 600 mg l^{-1} of the commercial formulate; the higher inertia percent on dimension 1 shows that these wines were preferred for their smoothness and yeast-like flavor.

This led us to think that the addition of yeast derivatives to non-aromatic white wines, like Pinot gris, could improve their sensory characters, by increasing their body; the perception of a low yeast-like note in this kind of wine may not be negative. Anyway the effect of dosage was not clear, and it would be very difficult to determine the optimal addition without doing a preliminary test.

Different behavior was observed for Traminer (but the same considerations could be made for Sauvignon): the preference of the panel was near the varietal aroma intensity, but quite far from the yeast-like perception; for this reason, the preferred sample was the control wine, without product addition. Anyway, the lowest additions (150 and 300 mg l^{-1}) seemed still to be related to aroma intensity, persistency, and smoothness of the wine, and this may confirm what was observed by olfactometry. The negative effects of too-high dosages could be particularly evident for varietal aromatic wines.

4. Conclusions

Yeast industrial derivatives demonstrated their ability to affect wine aroma perception with both direct and indirect effects: the former appear to be related to their flavoring properties and the consequent release of volatile compounds from the powders into the wine, the latter are probably connected to their ability to release soluble colloids and to affect the volatility of wine aroma compounds.

In this study, the dosage seemed to be a fundamental factor in the behavior of these two phenomena: the lowest addition determined an increase in the fruity and flowery perception of some volatile compounds (e.g. esters), while for higher amounts the release of some carboxylic acids characterized by cheese-like and unpleasant odors was observed.

In a first sensory approach, these last factors seemed not necessarily negative for the global perception of wine aroma, even if too-high dosages would not be suitable for wines with typical varietal aroma.

At this time, the characteristics of yeast derivatives available in the trade are still not well understood by wine-makers and there is often a lack in information from manufacturers as regards product composition. For these reasons, the olfactory examination of commercial formulates (to select less odorous and soluble products) and preliminary laboratory tests on small volumes could be useful tools to control the effects of the treatment.

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References

Ames, J. M., & Elmore, J. S. (1992). Aroma components of yeast extracts. *Flavour and Fragrance Journal, 7*, 89–103.

Ames, J. M., & McLeod, G. M. (1985). Volatile components of a yeast extract composition. *Journal of Food Science, 50*, 125–135.

Baek, H. H., & Cadwallader, K. R. (1999). Contribution of free and glycosidically bound volatile compounds to the aroma of muscadine grape juice. *Journal of Food Science, 64*, 441–444.

Barillere, J. M., & Benard, P. (1986). Exemples d'interprétation de résultats de dégustation. *Connaissance de la Vigne et du Vin, 20*(3), 137–154.

Comuzzo, P. (2003). Effetto di derivati industriali di lievito sulla stabilità colloidale e sulla percezione aromatica dei vini. Tesi di Dottorato, Università di Udine.

D'Agostino, A. (1990). Studio sull'utilizzo delle scorze di lievito e della cellulosa nel miglioramento della fermentazione birraria. Tesi di Laurea, Università di Udine.

Davidek, J., Hajslova, J., Kubelka, U., & Velisek, J. (1979). Flavour significant compounds in yeast autolysate Gistex X-II powder, Part 1. Acidic fraction. *Nahrung, 23*, 673–680.

Dufour, C., & Bayonove, C. L. (1999). Influence of wine structurally different polysaccharides on the volatility of aroma substances in a model system. *Journal of Agricultural and Food Chemistry, 47*, 671–677.

Fabietti, F., Delise, M., & Piccioli Bocca, A. (2000). Aromatic hydrocarbon residues in milk: preliminary investigation. *Food Control, 11*, 313–317.

Feuillat, M., Charpentier, C., & Nguyen Van Long, T. (1998). Les mannoprotéines de levures: un adjuvant œnologique possible. *Bulletin de l'O.I.V., 813–814*, 944–967.

Feuillat, M., Escot, S., Charpentier, C., & Dulau, L. (2001). Elevage des vins rouges sur lies fines – intérêt des interactions entre polysaccharides de levures et polyphénols du vin. *Revue des Œnologues, 98*, 17–18.

Fuster, A., & Escot, S. (2002). Elevage des vins rouges sur lies fines: choix de la levure fermentaire et ses conséquences sur les interactions polysaccharides pariétaux / polyphénols. *Revue des Œnologues, 104*, 20–22.

Grosch, W. (1987). Reactions of hydroperoxides-products of low molecular weight. In H. W.-S. Chan (Ed.), *Autoxidation of unsaturated lipids* (pp. 95–140). London: Academic Press.

Hajslova, J., Velisek, J., Davidek, J., & Kubelka, U. (1980). Flavour significant compounds in yeast autolysate Gistex X-II powder, Part 2. Neutral and basic fractions. *Nahrung, 24*, 875–881.

Jennings, W., & Shibamoto, T. (1980). *Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography*. New York: Academic Press.

Ledoux, V., Dulau, L., & Dubourdieu, D. (1992). Interprétation de l'amélioration de la stabilité protéique des vins au cours de l'élevage sur lies. *Journal International des Sciences de la Vigne et du Vin, 26*, 239–251.

Lopez, R., Ferreira, V., Hernandez, P., & Cacho, J. (1999). Identification of impact odorants of young red wines made with Merlot, Cabernet Sauvignon and Grenache grape varieties: a comparative study. *Journal of the Science of Food and Agriculture, 79*, 1461–1467.

Lubbers, S. (1993). Caractérisation de macromolécules d'origine levurienne du vin. Etude des interactions avec des substances d'arôme. Application a la stabilisation tartrique des vin. Thèse de Doctorat, Université de Bourgogne.

Lubbers, S., Charpentier, C., Feuillat, M., & Voilley, A. (1994a). Influence of yeasts walls on the behavior of aroma compounds in a model wine. *American Journal of Enology and Viticulture, 45*, 29–33.

Lubbers, S., Leger, B., Charpentier, C., & Feuillat, M. (1993). Effet collöide -protecteur d'extraits de parois de levures sur la stabilité tartrique d'une solution modèle. *Journal International des Sciences de la Vigne et du Vin, 27*(1), 13–22.

Lubbers, S., Voilley, A., Feuillat, M., & Charpentier, C. (1994b). Influence of mannoproteins from yeast on the aroma intensity of a model wine. *Lebensmittel – Wissenschaft und Technology, 27*(2), 108–114.

Moine Ledoux, V., Perrin, A., Paladin, I., & Dubourdieu, D. (1997). Premier résultats de stabilisation tartrique des vins par addition de mannoprotéines purifiées (Mannostab™). *Journal International des Sciences de la Vigne et du Vin, 31*(1), 23–31.

Münch, P., Hofmann, T., & Schieberle, P. (1997). Comparison of key odorants generated by thermal treatment of commercial and self-prepared yeast extracts: influence of the amino acid composition on odorant formation. *Journal of Agricultural and Food Chemistry, 45*, 1338–1344.

Münch, P., & Schieberle, P. (1998). Quantitative studies on the formation of key odorants in thermally treated yeast extracts using stable isotope dilution assays. *Journal of Agricultural and Food Chemistry, 46*, 4695–4701.

Nagodawithana, T. (1992). Yeast-derived flavors and flavor enhancers and their probable mode of action. *Food Technology, 46*(11), 138–144.

Nawar, W. W. (1969). Thermal degradation of lipids: a review. *Journal of Agricultural and Food Chemistry, 17*, 18–21.

Page, B. D., & Lacroix, G. (2000). Analysis of volatile contaminants in vegetable oils by headspace solid-phase microextraction with carboxen-based fibres. *Journal of Chromatography A, 873*, 79–94.

Piasenzotto, L., Gracco, L., & Conte, L. (2003). SPME applied to honey quality control. *Journal of the Science of Food and Agriculture, 83*, 1037–1044.

Regenstein, J. M., & Regenstein, C. E. (1984). *Food protein chemistry*. New York: Academic Press.

Saucier, C., Glories, Y., & Roux, D. (2000). Interactions tanins-collöides: nouvelles avancées concernant la notion de bons'' et de ''mauvais'' tanins. *Revue des Œnologues, 94*, 9–10.

Saucier, C., Roux, D., & Glories, Y. (1996). Stabilité collöidale de polymères catéchiques - influence des polysaccharides. In *Proceedings of Œnologie 95 – V Symposium International d'Œnologie de Bordeaux* (pp. 395–400). Bordeaux: Lavoisier Tec&Doc.

Schreier, P., Drawert, F., & Junker, A. (1977). The quantitative composition of natural and technologically changed aromas of plants. IV. Enzymic and thermal reaction products formed during the processing of tomatoes. *Zeitschrift fuer Lebensmittel-Untersuchung und-Forschung, 165*, 23–27.

Streitwieser, A., Heathcock, C. H., & Kosower, E. M. (1992). *Introduction to organic chemistry*. New York: Macmillan Publishing Company.

Tat, L., Comuzzo, P., Stolfo, I., & Battistutta, F. (2005). Optimization of wine headspace analysis by solid-phase microextraction capillary gas chromatography with mass spectrometric and flame ionization detection. *Food Chemistry, 93*, 361–369.

Tombesi, N. B., & Freije, H. (2002). Application of solid-phase microextraction combined with gas-chromatography-mass spectrometry to the determination of butylated hydroxytoluene in bottled drinking water. *Journal of Chromatography A, 963*, 179–183.

Usseglio-Tomasset, L., & Castino, M. (1975). I colloidi solubili di natura glucidica dei mosti e dei vini. *Parte I. Rivista di Viticoltura ed Enologia, 28*, 374–391.

Vichi, S., Pizzale, L., Conte, L. S., Buxaderas, S., & López-Tamames, E. (2003). Solid-phase microextraction in the analysis of virgin olive oil volatile fraction: modifications induced by oxidation and suitable markers of oxidative status. *Journal of Agricultural and Food Chemistry, 51*, 6564–6571.

Voilley, A., Beghin, V., Charpentier, C., & Peyron, D. (1991). Interaction between aroma substances and macromolecules in a model wine. *Lebensmittel – Wissenschaft und Technology, 24*, 469–472.

Voilley, A., Lamer, C., Dubois, P., & Feuillat, M. (1990). Influence of macromolecules and treatments on the behavior of aroma compounds in a model wine. *Journal of Agricultural and Food Chemistry, 38*, 248–251.

Waters, E. J., Wallace, W., Tate, M. E., & Williams, P. J. (1993). Isolation and partial characterization of a natural haze protective factor from wine. *Journal of Agricultural and Food Chemistry, 41*, 724–730.

Werkhoff, P., Bretschneider, W., Emberger, R., Guntert, M., Hopp, R., & Kopsel, M. (1991). Recent developments in the sulfur flavor chemistry of yeast extracts. *Chemie Mikrobiologie Technologie der Lebensmittel, 13*, 30–57.